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Technical/Final Report
to
Saskatchewan Agriculture Research Fund

Development of subunit vaccines for control of Bovine herpesvirus-1 (BHV-1) -induced respiratory disease

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Project title:

Development of a subunit vaccine for control of bovine herpesvirus-1 (Infectious Bovine Rhinotracheitis) induced respiratory disease (shipping fever).

Project manager:

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Organization:

Veterinary Infectious Disease Organization

University of Saskatchewan

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March 29, 1989

a) Executive Summary

I. Abstract

Bovine herpesvirus-1 (BHV-1) causes a number of diseases in cattle including respiratory disease, abortion and neonatal deaths. This virus consists of at least 30 proteins. We have shown that three of these proteins (gI, gIII and gIV) are responsible for formation of protective immunity against the virus. To develop a commercial vaccine, it is necessary to produce these glycoproteins in large amounts. The baculovirus system was chosen for this purpose because of its abundant expression of recombinant proteins from foreign genes. Preliminary data indicate that glycoproteins gI and gIII are expressed at reasonable to high levels, whereas gIV is expressed abundantly in recombinant baculovirus infected cells.

II. Summary

Bovine herpesvirus-1 (BHV-1) is one of the major initiators of Pneumonic Pasteurellosis, a disease that costs the North American cattle industry $250 million annually. It is thought that BHV-1 causes primary lesions in the respiratory tract as well as immunosuppression of the animal, which allows growth of the bacteria resulting in pneumonia and death. Thus, immunization of cattle against BHV-1 could reduce economic losses due to the disease. The objective of this project is to develop a subunit vaccine that in contrast to the commonly used live attenuated vaccines, is safe and efficient. BHV-1 contains at least 30 different proteins within the virion. In previous studies we were able to show that three of these proteins (gI, gIII and gIV) are responsible for the induction of protective immunity against the virus.

To develop a commercial vaccine, it is necessary to produce these glycoproteins in large amounts. Therefore, we are investigating different approaches to produce the BHV-1 glycoproteins on a large scale.

In the present study we demonstrate the abundant expression of glycoprotein gIV in recombinant baculovirus infected cells. Preliminary data indicate that glycoproteins gI and gIII are also expressed at reasonable to high levels in this system. We expect to test the protective capacity of these three recombinant-expressed glycoproteins in cattle in the near future.

In addition to baculovirus, we have explored alternative

systems for the expression of the BHV-1 glycoproteins. To date, gI, gIII and gIV have been expressed in transfected mammalian cells, and gIII is produced in recombinant adenovirus infected cells. However, these systems need to get optimized in order to achieve higher expression of the glycoproteins.

As an alternative to the recombinant DNA methods, we have started the propagation of BHV-1 in cells grown on microcarrier beads. Since the MDBK cells used for propagation of BHV-1 are growing well on the microcarrier beads at a density of 10^{10} cells/10 mg beads/10L run, this method also looks very promising, although we expect the purification of the glycoproteins from these cells to be more laborious and expensive than from recombinant virus-infected or transfected cells.

Finally, an improved method of immunizing cattle with vaccinia recombinant virus expressing gI or gIII resulted in the induction of serum neutralizing antibodies to BHV-1. The titers of these antibodies were comparable to those obtained after two immunizations of conventionally produced and purified gI and gIV, which indicates that the levels of the antibody titers were sufficient to induce protection from challenge with BHV-1.

In conclusion, the baculovirus-expressed glycoproteins gI, gIII and gIV appear to be very good candidates for a commercial subunit vaccine to BHV-1. In addition, several other alternative production methods are available. The benefits of having such a vaccine will be of value to the beef producer, dairy industry and purebred breeder interested in export of pedigree stock or semen.

b) Technical Report

I. Abstract

The objectives of this project were to test the protective capacity of bovine herpesvirus-1 (BHV-1) glycoproteins produced a) in a baculovirus expression system and b) in BHV-1 infected cells grown on microcarrier beads.

Glycoproteins gI and gIII were expressed in recombinant baculovirus infected cells, initially at very low levels. However, preliminary data indicate that these levels have been greatly increased. Glycoprotein gIV has been successfully expressed in baculovirus, resulting in the production of very high levels of gIV in recombinant baculovirus infected cells that exceed those in BHV-1 infected cells. These recombinant baculovirus derived glycoproteins will be tested for their ability to induce protective immunity to BHV-1 in cattle in the very near future.

The fermenters needed for the production of large amounts of BHV-1 infected MDBK cells grown on microcarrier beads have not been available yet. Consequently, although we have obtained excellent growth of MDBK cells on microcarrier beads at a density of 10^{10} cells/10 mg beads/10L run, as well as high-titered stock BHV-1 (10^{9} pfu/ml) for infection of these cells, we have not yet been able to test glycoproteins derived from microcarrier cultures in cattle.

Instead, we repeated a previously performed experiment, in which we immunized cattle with recombinant vaccinia virus expressing gI and gIII using an improved method of immunization. High titers of serum neutralizing antibodies to BHV-1 were induced,

which were comparable to those reached after two immunizations of affinity-purified gI or gIII, indicating the potential usefulness of vaccinia virus for the development of a vaccine against BHV-1.

II. Background and Objectives

Bovine herpesvirus type-1 (BHV-1) is an important pathogen of cattle, which causes severe respiratory infections as well as conjunctivitis, vulvovaginitis, abortions, encephalitis, and generalized systemic infections (Gibbs and Rweyemamu, 1977). Mechanisms by which animals recover from virus infections include cell-mediated as well as humoral immune responses (Rouse and Babiuk, 1978). Virus-infected cells may be destroyed by antibody-dependent cellular cytotoxicity (ADCC) (Rouse et al., 1975, 1976; Grewal et al., 1977) and T cell-mediated cytotoxicity (Rouse and Babiuk, 1977) or antibody-dependent, complement-mediated (AbC') cytolysis (Babiuk et al., 1975). Outside the cells, virions can be neutralized by antibody or lysed by antibody and complement (Babiuk et al., 1975).

Like most herpesviruses, BHV-1 induces latency after primary infection (Gibbs and Rweyemamu, 1977; Babiuk and Rouse, 1979; Homan and Easterday, 1980). It is believed that the virus, like other herpesviruses, can remain latent in neurological cells (Stevens, 1978) and can be reactivated after dexamethasone treatment (Sheffy and Davies, 1972; Pastoret et al., 1979). Furthermore, not only wild-type virus, but also live attenuated BHV-1 vaccine can become latent and can be reactivated by dexamethasone (Pastoret et al., 1979). Since control of BHV-1 is based largely on vaccination, and

the safety of the commonly used live attenuated vaccines has been questioned, subunit vaccines, consisting of the immunodominant proteins, would be considered as a viable alternative. Due to the availability of recombinant DNA technology and the feasibility for chemical synthesis of short peptides of specific amino acid sequences, considerable progress has been made toward the production of subunit vaccines (Kleid et al., 1981; Bittle et al., 1982; Cohen et al., 1984).

For the preparation of a BHV-1 subunit vaccine, more information regarding the polypeptides and mechanisms involved in immune responses against BHV-1 is required. Recently we have begun to analyze the polypeptides of BHV-1 in order to determine their antigenic and immunogenic properties. Using monoclonal antibodies, four unique glycoproteins have been identified: gI, gII, gIII and gIV (van Drunen Littel-van den Hurk et al., 1984; van Drunen Littel-van den Hurk and Babiuk, 1986b).

Monoclonal antibodies against these major glycoproteins participated to different extents in virus neutralization and antibody- and complement-mediated (AbC') cytolysis of virus-infected cells. In addition, purified gI, gIII and gIV stimulated the production of monospecific antibodies in rabbits, which neutralized viral infectivity and mediated immune lysis of virus-infected cells (van Drunen Littel-van den Hurk and Babiuk, 1985). These individual glycoproteins also stimulated antibody production in cattle infected with whole virus (van Drunen Littel-van den Hurk and Babiuk, 1986a) and, most importantly, they were all able to

protect cattle from lethal BHV-1/Pasteurella haemolytica challenge (Babiuk et al, 1987).

To develop a commercial vaccine employing one or more of these glycoproteins, they have to be produced in large amounts. The objective of this proposal was to test the protective capacity of these glycoproteins produced by two different methods: a) recombinant DNA proteins produced in a baculovirus expression system, and b) natural proteins purified from virus grown on microcarriers.

In this report we describe and show the abundant expression of BHV-1 glycoprotein gIV in recombinant baculovirus-infected cells. Since this has only just been achieved, we have as yet been unable to test this recombinant protein in cattle, but we will perform this experiment in the very near future.

At present the MDBK cells used for propagation of BHV-1 are growing well on the microcarrier beads. In addition, high-titered stock virus, needed to infect microcarrier-grown MDBK cells, has been produced. However, the fermenters needed for the large-scale production of BHV-1 infected MDBK cells grown on microcarriers have not been available yet.

However, we have proceeded to test recombinant BHV-1 glycoproteins in cattle and chosen gI and gIII expressed by vaccinia recombinant virus as an alternative source, using an improved immunization method.

IV. Experimental Methods

a) Production of recombinant baculovirus expressing BHV-1

glycoproteins

The cloned coding sequences for BHV-1 glycoproteins gI, gIII and gIV were inserted into the BAM HI site of baculovirus transfer vector pVL941 (Luckow and Summers, 1988). The genes were inserted into the genome of Autographa californica nuclear polyhedrosis (ANPV) virus by homologous recombination in ANPV-infected SF-9 cells (Summers and Smith 1987). Recombinant viruses were identified by plaque hybridization followed by three cycles of plaque purification.

Production of recombinant polypeptide was assayed by radioimmunoprecipitation of extracts of virus-infected SF-9 cells at 48 hours post-infection.

SDS - PAGE - Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 8.5% polyacrylamide discontinuous gels (Laemmli, 1970) as described previously (van Drunen littel-van den Hurk et. al., 1984).

Western blot analysis - The Western blotting technique of Burnette (1981) was used as previously described (van Drunen littel-van den Hurk et. al., 1984). Briefly, after electrophoresis, cell lysates and purified glycoproteins were electrophoretically transferred to nitrocellulose sheets using 25mM sodium phosphate buffer (pH 6.8) as electrode solution. The nitrocellulose was then incubated with a blocking solution of 3% blotto in 0.02 M Tris-hydrochloride, 0.5 M NaCl, pH 7.5 (TBS), followed by incubation with a 1:1000 dilution of monoclonal ascites fluids in TBS containing 1% blotto. Finally, the nitrocellulose was incubated with a 1:1000 dilution of

affinity-purified, horseradish peroxidase (HRPO) conjugated rabbit anti-mouse IgG and developed with the BioRad development solution containing 4-chloronaphthol.

Enzyme-linked immunosorbent assay - A sandwich ELISA was used to compare the yields of glycoproteins gI and gIV from recombinant-infected cells to those from BHV-1 infected cells. Microtiter plates were coated with 200 ul per well of a mixture of monoclonal IgG as the captive antibody and then incubated with 200 ul per well of lysate from recombinant- or BHV-1-infected cells. Cell lysates were prepared by suspending 1.5×10^7 cells in 1.5 ml of detergent-containing buffer. The cells were left on ice for 15 min and sonicated for 15 s at a setting of 100 on a Sonifier cell disrupter before use in the ELISA. A mixture of HRPO-conjugated monoclonal antibodies with a different epitope specificity was used for detection at 200 ul per well.

b) Cells and virus - Strain P8-2 of BHV-1 was propagated in Madin Darby Bovine Kidney (MDBK) cells, using modified Eagle's medium (MEM) and 10% fetal bovine serum (FBS).

c) Production of recombinant vacciniavirus expressing gI (VAC-I) or gIII (VAC-III) - The complete coding sequence of BHV-1 was excised from a subclone of psD106 (Mayfield et. al., 1983) and inserted into the BAM H1 site of pGS20 (Moss et. al., 1983). This construction placed the BHV-1 gI gene sequence immediately downstream of the vacciniavirus 7.5 kD promoter, which is flanked by vaccinia's thymidine kinase gene sequences. This construction was transfected into BSC-40 cells infected with vaccinia (strain

Wyatt or strain WR) and recombinant virus was selected by passage into the thymidine kinase-negative 43 cells growing in bromodeoxyuridine medium. The progeny of individual plaques was screened for expression of gI by ELISA using polyclonal or monoclonal antibodies specific for BHV-1 gI.

Recombinant vacciniavirus expressing BHV-1 gIII was produced in a similar manner except the complete coding sequence of gIII was derived from a subclone of pSD113 (Mayfield et. al., 1983).

Immunization of cattle - Groups of three to five calves each were immunized by intradermal (i.d.) as well as intramuscular (i.m.) inoculation with VAC-I, VAC-III or VAC. The i.d. inoculation consisted of 10^8 pfu of virus in 1 ml, whereas the i.m. inoculation contained 10^7 pfu in a 10 ml-volume. The animals were boosted twice at 21-day intervals with an equal amount of virus. Animals were bled weekly for assessment of antibody responses.

Neutralization test - The neutralization titers of the bovine sera were determined as described previously (Babiuk et. al., 1975) and expressed as the reciprocal of the dilution causing a 50% reduction of plaques relative to the virus control.

Enzyme-linked immunosorbent assays - In order to identify recombinant vaccinia virus, expressing gI or gIII, an indirect ELISA was performed essentially as described previously (van Drunen Littel-van den Hurk et. al., 1984). Microtiter plates were coated with cell extracts prepared from recombinant TK^- virus-infected BSC-40 cells and reacted with gI- or gIII-specific rabbit sera. Affinity-purified, HRPO-conjugated goat anti-rabbit IgG

(Boehringer-Mannheim, Dorval, Quebec) was used at a dilution of 1:2000 for detection.

A sandwich ELISA was used to compare the yields of glycoproteins gI and gIII from recombinant infected cells to those from BHV-1 infected cells, as described under section a).

In order to determine the antibody responses of cattle immunized with the recombinant vaccinia viruses, the ELISA was performed essentially as described previously (van Drunen Littel-van den Hurk et. al., 1984). However, affinity-purified, HRPO-conjugated rabbit anti-bovine IgG (Zymed) at a dilution of 1:3000 was used as the detecting antibody.

IV. Results and Discussion

a) Production of recombinant BHV-1 glycoproteins in a baculovirus expression system

One of the major advantages of the baculovirus system is the abundant expression of recombinant proteins from foreign genes. In collaboration with scientists at the Biotechnology Research Institute of the NRC, we have been working to clone BHV-1 genes into this system. The genes for BHV-1 glycoproteins gI and gIII have been inserted into the baculovirus genome and several recombinant viruses containing these genes have been tested at VIDO for expression of glycoproteins gI and gIII. Unfortunately, hardly any recombinant gIII was detected (data not shown), whereas small amounts of recombinant gI were found both by Western blotting and ELISA. However, the quantities of recombinant gI were lower than those produced by equivalent numbers of BHV-1 infected Madin Darby

bovine kidney (MDBK) cells, which are routinely used for the production of BHV-1 glycoproteins (Fig. 1 and Table 1). Since the purpose of expression in the baculovirus system was to minimize the expense of producing these BHV-1 glycoproteins, we have been concentrating our efforts at VIDO on increasing the levels of expression of glycoproteins gI and gIII in baculovirus infected cells. Preliminary data indicate that gI and gIII are now being expressed at reasonable to high levels (data not shown). In addition, we have achieved abundant expression of gIV in this system (Fig. 2 and Table 1). Since the expression of these BHV-1 glycoproteins, and glycoproteins in general, has proven to be more difficult than the production of regular, unglycosylated proteins, resulting in unexpected delays, we have as yet been unable to test the baculovirus-expressed recombinant glycoproteins in cattle, but we hope to perform this experiment in the very near future.

In addition, even though we prefer to use the baculovirus expression system, because of its generally abundant expression of foreign (glyco) proteins, we are investigating alternative expression systems. To date, gI, gIII and gIV have been expressed in transfected mammalian cells and gIII is produced in recombinant adenovirus-infected cells. At the present time we are working on optimizing these systems in order to achieve more abundant expression of these glycoproteins.

b) <u>Production of BHV-1 glycoproteins in MDBK cells grown on microcarriers</u>

Using the microcarrier technology, tissue culture cells can

be grown at much higher density than with conventional methods. Consequently, BHV-1 glycoproteins are produced more economically in cells grown on microcarrier beads. At present, the MDBK cells used for propagation of BHV-1 are growing well on the microcarrier beads at a density of 10^{10} cells/10 mg beads/10L run. In addition, high-titered stock virus (10^{9} pfu/ml) needed to infect microcarrier-grown MDBK cells, has been produced. In order to grow the cells in high quantities, we need fermenters, which thus far have not been available. As soon as they are, we will proceed to prepare large amounts of BHV-1 infected MDBK cells on microcarrier beads, and purify the glycoproteins from these cells to be tested in cattle.

c) Immunogenicity of BHV-1 glycoproteins produced in a vaccinia recombinant virus system

Previously, we reported on the insertion of the genes for glycoproteins gI and gIII into the vaccinia virus genome. *In vitro* experiments indicated that the products of the BHV-1 glycoprotein genes were similar in vaccinia recombinant and BHV-1 infected cells. However, the levels of glycoproteins gI and gIII produced by vaccinia virus were approximately 6-fold lower than those produced by BHV-1 (Table 2). Initial experiments *in vivo* indicated that these vaccinia recombinant viruses were not able to stimulate glycoprotein-specific neutralizing antibodies in cattle. Recently, we repeated these experiments using an improved method of immunization. Three groups of calves were inoculated with VAC-I, VAC-III or VAC. Following two immunizations, animals immunized

with VAC-I or VAC-III developed a high titer of serum neutralizing antibodies to BHV-1 (Fig. 3). Following a third immunization, there was no further increase in the level of serum neutralizing antibodies to BHV-1 induced by the recombinant viruses. The sera of the animals that were inoculated with VAC did not show any neutralizing activity. To further measure the specificity of the immune response, the sera were tested in an ELISA, using either gI or gIII as the antigen. Figure 4 shows that animals immunized with VAC-I developed antibodies to gI, whereas animals inoculated with VAC-III recognized gIII, indicating that the virus neutralizing activity was specific for the respective glycoproteins. Animals that received VAC did not react with either gI or gIII. Although *in vitro* the level of expression of recombinant gI and gIII appeared to be lower than that of their authentic counterparts, it was sufficient for the induction of serum neutralizing antibodies in cattle. These antibodies recognized authentic gI and gIII confirming the specificity of the neutralizing antibody response. It is important to note that the recombinant glycoproteins gI and gIII, even though in competition with numerous vaccinia antigens, induced neutralizing antibody titers comparable to those reached after two immunizations of affinity-purified authentic gI and gIII (Babiuk et. al., 1987). Previously, the level of these titers was found to be high enough for the induction of protection against challenge with BHV-1 and *Pasteurella haemolitica* in cattle (Babiuk et. al., 1987), indicating the potential usefulness of vaccinia virus for the development of a vaccine against BHV-1.

IV. Conclusions and Impact

Respiratory disease in cattle causes major economic losses annually to the Saskatchewan and Western Canadian cattlemen. One of the major initiators of the disease is BHV-1, commonly called IBRV. In addition to causing respiratory disease, this virus also causes abortion in cattle and can be transmitted in semen. Therefore, countries free of BHV-1 refuse to accept semen from animals that have antibodies to the virus, because these animals could be potential carriers of BHV-1. Control of BHV-1 is based largely on vaccination, though the safety and efficacy of the commonly used live attenuated vaccines have been questioned. Therefore we intend to develop a subunit vaccine, consisting of the immunodominant proteins of BHV-1. Previously we demonstrated that as little as two doses of 6.25 ug of a purified glycoprotein gI, gIII or gIV induced protection against challenge with BHV-1, suggesting that the individual glycoproteins may each be useful candidates for a subunit vaccine against BHV-1. However, even though we only needed 12.5 ug of glycoprotein per animal, perhaps even less, the production of such a vaccine by conventional methods is not economical. Therefore, we have been investigating different approaches to produce the BHV-1 glycoproteins on a large scale.

In the present study we demonstrated the abundant expression of glycoprotein gIV in recombinant baculovirus infected cells. In addition, the expression of glycoprotein gI and gIII also appeared to be much improved over previously obtained amounts. Consequently, we expect to test these three recombinant

glycoproteins in cattle in the very near future.

Since the expression of these large glycoproteins in baculovirus appeared to be more difficult than we anticipated, we have been exploring alternative systems. To date, gI, gIII and gIV have been expressed in transfected mammalian cells and gIII is produced in recombinant adenovirus infected cells. However, these systems need to get optimized for more abundant expression of the glycoproteins.

As an alternative to the recombinant DNA methods, we have explored the propagation of BHV-1 in cells grown on microcarriers. Since the MDBK cells, used for propagation of BHV-1, are growing well on the microcarrier beads at a density of 10^{10} cells/10 mg beads/10L run, this method also looks very promising, although we expect the purification of the glycoproteins to be more laborious and more expensive than from recombinant virus-infected or transfected cells.

Finally, an improved method of immunizing cattle with vaccinia recombinant virus expressing gI or gIII resulted in the induction of neutralizing antibodies to BHV-1. The titers of these antibodies were comparable to those reached after two immunizations with affinity-purified gI or gIII. The levels of these titers were found to be high enough to induce protection from challenge with BHV-1 (Babiuk et. al., 1987).

In conclusion, the baculovirus-expressed glycoproteins gI, gIII and gIV appear to be very good candidates as a subunit vaccine to BHV-1. In addition, several other production methods are

available. The benefits of having such a vaccine will be of value to the beef producer, dairy industry and purebred breeder interested in export of pedigree stock or semen.

Table 1. Comparison of the amounts of glycoproteins gI and gIV synthesized in recombinant and BHV-1 infected cells.

	ELISA titer[a] in cell line:			
	MDBK		SF9	
Virus	gI	gIV	gI	gIV
BHV-1	2560	1024	ND	ND
Baculovirus-gI	ND[b]	ND	160	ND
Baculovirus-gIV	ND	ND	ND	4096

[a] Recombinant and BHV-1 infected cells were lysed in detergent-containing buffer and microtiter plates, coated with 200 ul per well of monoclonal IgG were incubated with 200 ul per well of cell lysate. Reaction was detected by incubation with 200 ul per well of HRPO-conjugated monoclonal antibodies. ELISA titers were expressed as the reciprocal of the highest dilution that still gave a reading of at least 0.1 OD.

[b] N.D. = not done

Table 2. Comparison of the amounts of glycoproteins gI and gIII synthesized in recombinant and BHV-1 infected cells.

	ELISA Titer[a] in Cell Line:							
	BSC-1		MDBK		BFB		BTB	
Virus	gI	gIII	gI	gIII	gI	gIII	gI	gIII
None	16	16	4	16	16	16	4	4
BHV-1	102	42	2000	1200	170	25	256	48
VAC	4	4	4	16	4	4	<4	4
VAC-I	320	4	16	16	200	4	85	4
VAC-III	<4	190	4	16	4	30	<4	25

a Recombinant- or BHV-1-infected cells were lysed in detergent-containing buffer and microtiter plates, coated with 200 ul per well of monoclonal IgG were incubated with 200 ul per well of cell lysate. Reaction was detected by incubation with 200 ul per well of HRPO-conjugated monoclonal antibodies. ELISA titers were expressed as the reciprocal of the highest dilution that still gave a reading of at least 0.1 OD.

Figure Legends

Figure 1

When recombinant DNA proteins are produced in an expression system such as baculovirus, it is necessary to detect the protein and confirm its identity. This can be done using a technique referred to as Western Blotting which is shown in this figure. Glycoprotein gI (conventional and recombinant) was separated from a number of other proteins by polyacrylamide gel electrophoresis and transferred to nitrocellulose paper. Its presence was confirmed by incubation of the paper with a mixture of gI-specific monoclonal antibodies, followed by an enzyme-labelled secondary antibody and a substrate that visualizes the gI-antibody complex on the paper. A: BHV-1 infected MDBK cells; B to G: baculo gI-recombinant infected SF9 cells at 0, 24, 48, 72, 96, 120 h post-infection; H: purified gI.

Figure 2

Detection of conventional and recombinant gIV by Western blotting. Glycoprotein gIV was separated from a number of other proteins by polyacrylamide gel electrophoresis and transfered to nitrocellulose paper. Its presence was confirmed by incubation of the paper with a mixture of gIV-specific monoclonal antibodies, followed by an enzyme-labelled secondary antibody and a substrate that visualizes the gIV-antibody complex on the paper. A: Mock-infected MDBK cells; B: BHV-1 infected MDBK cells; C: Mock-infected SF9 cells; D to F: baculo gIV-recombinant infected SF9 cells at 23, 44 and 68 h post-infection: G: purified gIV.

Figure 3

Serum neutralizing antibody responses to BHV-1 of animals immunized with VAC recombinants expressing gI (VAC-I; ●) or gIII (VAC-III; □) or with VAC (O). The animals received booster immunizations 3 and 6 weeks later. Titers were determined by a 50% endpoint using 100 PFU of BHV-1.

Figure 4

ELISA titers of animals immunized with VAC-I (●), VAC-III(□), or VAC (O). The animals received booster immunizations 3 and 6 weeks later. The microdilution plates were coated with gI (o.25 ul/ml; ●), gIII (0.25 ug/ml; □), or gI plus gIII (0.25 ug/ml each; O). The ELISA titer is expressed as the reciprocal of the dilution that still resulted in a reading of 0.1.

c) Papers and talks

S. van Drunen Littel-van den Hurk, Zamb, T. and Babiuk, L.A. 1989. Synthesis, cellular location and immunogenicity of bovine herpesvirus-1 glycoproteins expressed by recombinant vacciniavirus. J. Virol. In Press.

d) Statement of expenditures will be forwarded separately.

e) Research technician 10%

Research assistant 5%

Animal technician 5%

Involvement of scientists

f) No equipment of item value in excess of $500 has been purchased.

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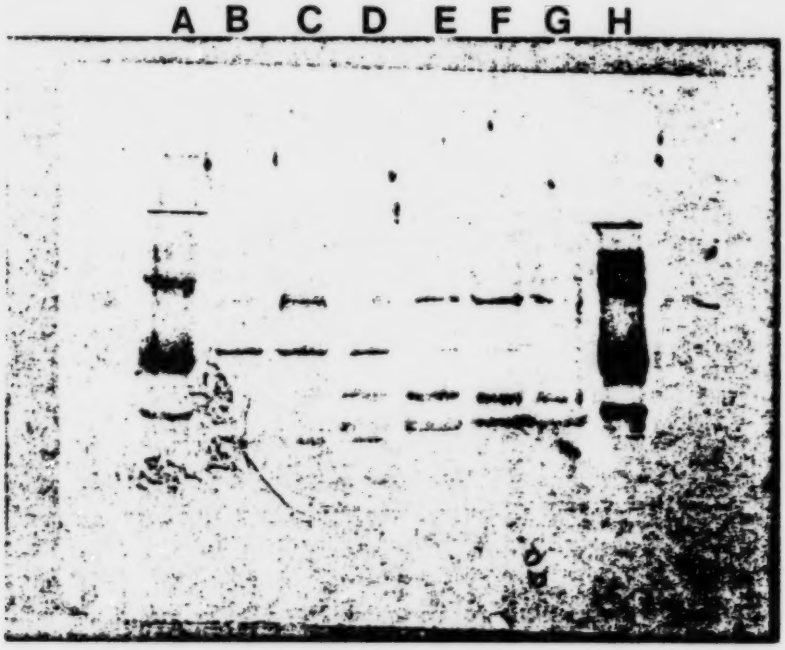


Figure 1

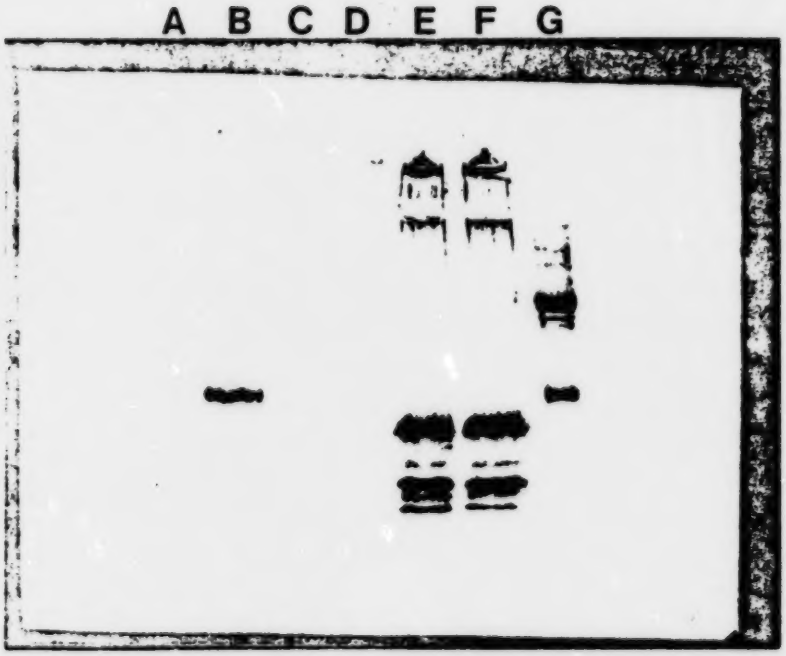
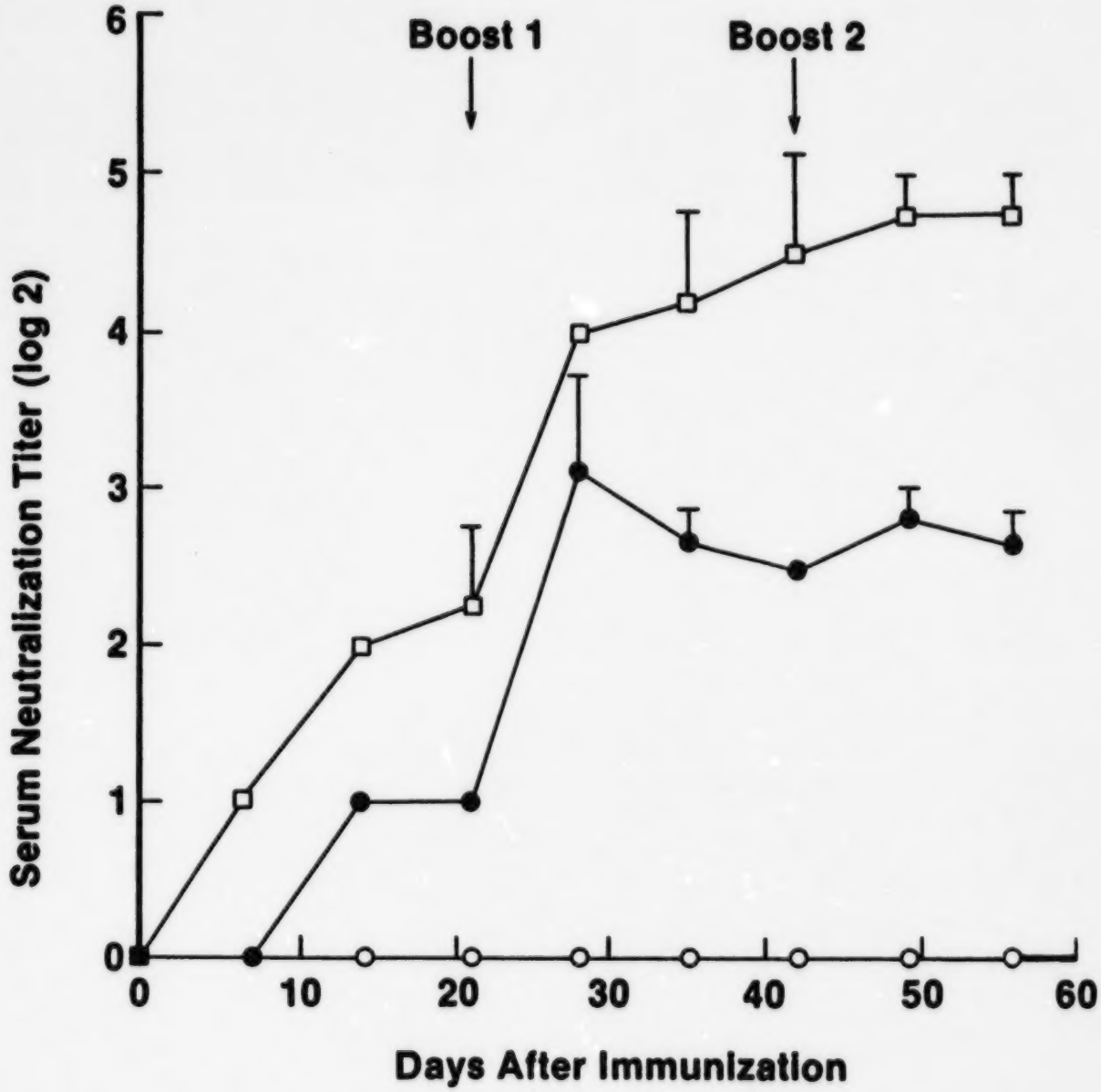


Figure 2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Boost 2
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2
3
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40
50
60
Days After Immunization

Figure 3

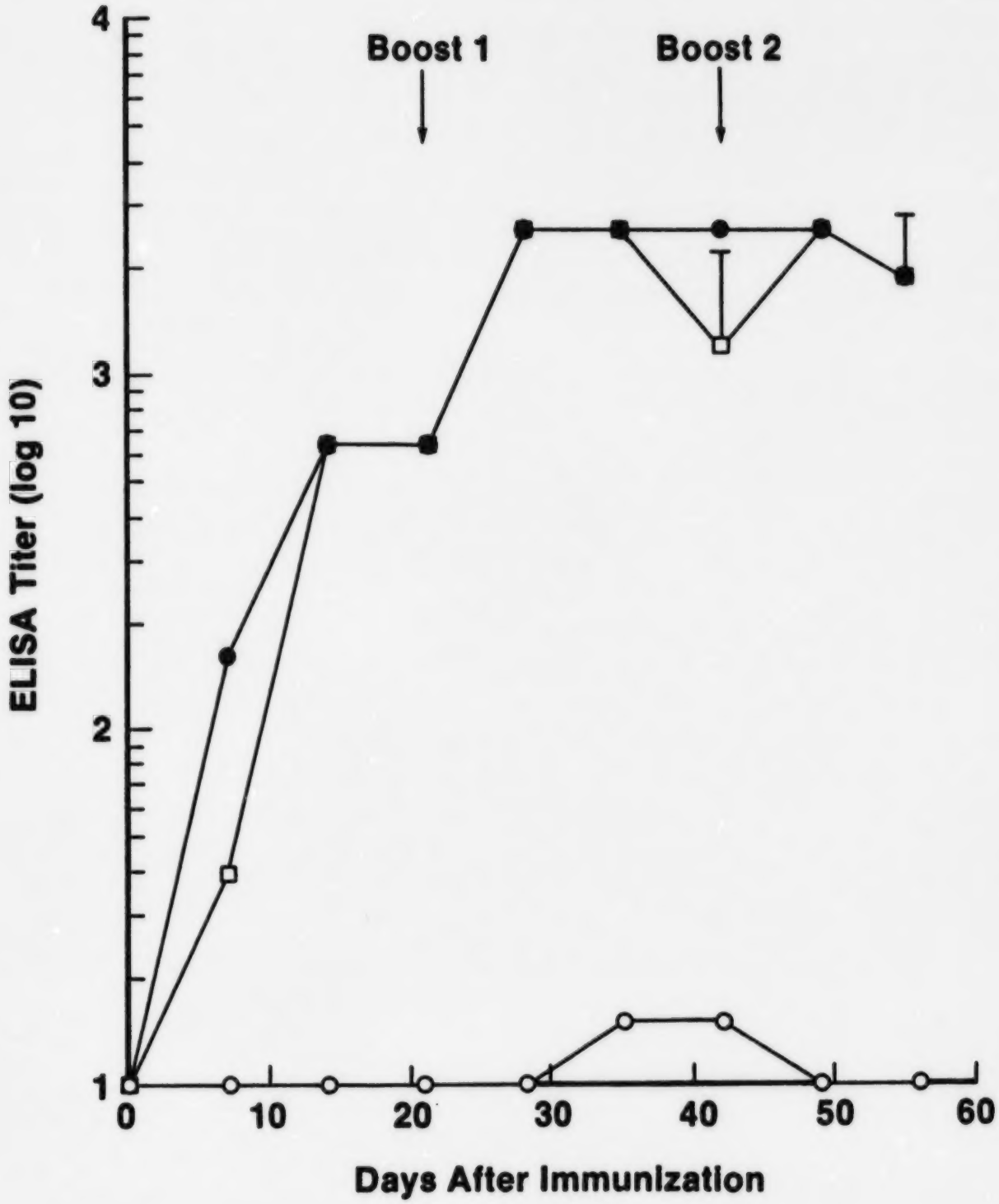
Boost 1
Boost 2
ELISA Titer (log 10)
4
3
2
1
0
10
20
30
40
50
60
Days After Immunization

Figure 4

5-85-09

Project Title:

Development of subunit vaccines for control of Bovine herpesvirus-1 (BHV-1) (Infectious Bovine Rhinotracheitis - IBR) - induced respiratory disease (shipping fever).

Project Manager:

S. van Drunen Littel-van den Hurk

Organization:

Veterinary Infectious Disease Organization
University of Saskatchewan

Date of Submission:

July 31, 1987

Summary

Bovine herpesvirus-1 (BHV-1) is one of the major initiators of Pneumonic Pasteurellosis, a disease that costs the North American cattle industry $250 Million annually. It is thought that BHV-1 causes primary lesions in the respiratory tract as well as immunosuppression of the animal, which allows growth of the bacteria resulting in pneumonia and death. Thus, immunization of cattle against BHV-1 could reduce economic losses due to the disease. The objective of this project is to develop a subunit vaccine that, in contrast to the commonly used live attenuated vaccines, is safe and efficient.

BHV-1 has over 30 different proteins within the virus, of which only a few may be involved in inducing protection from infection. We have developed monoclonal antibodies and identified the three important glycoproteins on the surface of BHV-1 that are involved in neutralizing the virus (preventing infection). We then used the monoclonal antibodies to purify these glycoproteins gI, gIII and gIV by immunoadsorbent chromatography. Rabbits were immunized with each of these purified glycoproteins and they produced an immune response. The sera from these rabbits neutralized the virus, suggesting that these glycoproteins are potential candidates for a subunit vaccine.

In this project the ability of each of these three glycoproteins to protect cattle from lethal BHV-1/Pasteurella haemolytica (P. Haem.) challenge was assessed and compared with that of a killed commercially available vaccine, Triangle 3, and a placebo. After two immunizations with affinity-purified glycoproteins gI, gIII or gIV the calves developed high titers of antibody specific for the respective immunogens. These antibodies mediated virus neutralization and destruction of virus-infected cells. The clinical response to challenge was significantly lower in the animals immunized with the purified glycoproteins than in those immunized with Triangle 3 or a placebo. Accordingly, whereas the survival rates in the placebo and conventional vaccine groups were only 40-60%, all animals vaccinated with gI, gIII or gIV, or any combination thereof, were protected.

In order for a subunit vaccine to be economical in use, it has to be produced in large quantities at minimal expense. One system involves the expression of the glycoprotein genes in vacciniavirus. The genes for glycoproteins gI and gIII have each been inserted successfully into the vacciniavirus genome. However, neither of the two recombinant vacciniaviruses were able to stimulate the production of high levels of glycoprotein-specific antibodies or to confer protection to lethal BHV-1/P. haem. challenge in cattle. Consequently, we are now exploring different methods for large-scale glycoprotein purification.

Once a subunit vaccine has been developed, it will be important to differentiate animals vaccinated with a particular glycoprotein (subunit), from those immunized with conventional vaccine or infected with a field strain. To this end, we developed an enzyme-linked immunosorbent assay (ELISA) using purified glycoproteins as test antigens. With the assay we can detect whether animals have antibody to just the glycoprotein(s) used in the vaccine, or whether they also have antibodies to other BHV-1 proteins. If the latter is the case, then the animals have been infected with live virus and can be potential carriers of the virus. If they only have antibody to the glycoprotein(s) used for the vaccination, then the animals should be protected from the disease, but are not carriers.

As mentioned above, respiratory disease is a major problem for Saskatchewan and Western Canadian cattlemen. One of the major initiators is

BHV-1. In addition to causing respiratory disease, this virus also causes abortions in cattle and can be transmitted in semen. Therefore, countries free of BHV-1, are refusing to accept semen from animals that have antibodies to the virus, because these animals could be potential carriers of the virus. Thus, the potential benefits of having a subunit vaccine and a test to differentiate animals vaccinated with subunit vaccine will be of value to the beef producers, dairy industry and purebred breeders interested in export of pedigree stock or semen.

Abstract

The objectives of this project were to 1) test the ability of affinity-purified BHV-1 glycoproteins to prevent respiratory disease in cattle; 2) test the protective capacity of BHV-1 glycoproteins produced by recombinant vacciniavirus; and 3) develop an enzyme-linked immunosorbent assay (ELISA) to differentiate animals vaccinated with individual glycoproteins from those carrying virus.

The major glycoproteins gI, gIII and gIV of BHV-1 were found to induce high levels of antibody in cattle which could neutralize virus and participate in antibody-dependent cell cytotoxicity of BHV-1-infected cells. Immunized animals were fully protected from disease, using a BHV-1/Pasteurella haemolytica aerosol challenge model. These results suggest that any one of the three major BHV-1 glycoproteins may be useful as a subunit vaccine either individually or in combination.

In order for a subunit vaccine to be economical in use, it has to be produced in large quantities at minimal expense. One potential system involves the expression of the glycoprotein genes in vacciniavirus. The genes for glycoproteins gI and gIII have each been successfully inserted into the vacciniavirus genome. However, neither of the two recombinant vacciniaviruses were able to stimulate the production of high levels of glycoprotein-specific antibodies or to confer protection to lethal BHV-1/Pasteurella haemolytica challenge in cattle.

The sera from the vaccinated animals were tested in an ELISA using purified glycoproteins as test antigens. The specificity of the test was demonstrated by the observation that animals immunized with a particular glycoprotein reacted with that glycoprotein only, but not with any of the others. However, animals vaccinated with conventional vaccine reacted with all glycoproteins.

II. Background and Objectives

Bovine herpesvirus type-1 (BHV-1) is an important pathogen of cattle, which causes severe respiratory infections as well as conjunctivitis, vulvovaginitis, abortions, encephalitis, and generalized systemic infections (Gibbs and Rweyemamu, 1977). Mechanisms by which animals recover from virus infections include cell-mediated as well as humoral immune responses (Rouse and Babiuk, 1978). Virus-infected cells may be destroyed by antibody-dependent cellular cytotoxicity (ADCC) (Rouse et al., 1975, 1976; Grewal et al., 1977) and T cell-mediated cytotoxicity (Rouse and Babiuk, 1977) or antibody-dependent, complement-mediated (AbC') cytolysis (Babiuk et al., 1975). Outside the cells, virions can be neutralized by antibody or lysed by antibody and complement (Babiuk et al., 1975).

Like most herpesviruses, BHV-1 induces latency after primary infection (Gibbs and Rweyemamu, 1977; Babiuk and Rouse, 1979; Homan and Easterday, 1980). It is believed that the virus, like other herpesviruses, can remain latent in neurological cells (Stevens, 1978) and can be reactivated after dexamethasone treatment (Sheffy and Davies, 1972; Pastoret et al., 1979). Furthermore, not only wild-type virus, but also live attenuated BHV-1 vaccine can become latent and can be reactivated by dexamethasone (Pastoret et al., 1980). Since control of BHV-1 is based largely on vaccination, and the safety of the commonly used live attenuated vaccines has been questioned, subunit vaccines, consisting of the immunodominant proteins, would be considered as a viable alternative. Due to the availability of recombinant DNA technology and the feasibility for chemical synthesis of short peptides of specific amino acid sequences, considerable progress has been made toward the production of subunit vaccines (Kleid et al., 1981; Bittle et al., 1982; Cohen et al., 1984).

For the preparation of a BHV-1 subunit vaccine, more information regarding the polypeptides and mechanisms involved in immune responses against BHV-1 is required. Recently we have begun to analyze the polypeptides of BHV-1 in order to determine their antigenic and immunogenic properties. Using monoclonal antibodies, four unique glycoproteins have been identified: gI, gII, gIII and gIV (Misra et al., 1982; van Drunen Littel-van den Hurk et al., 1984; van Drunen Littel-van den Hurk and Babiuk, 1986a).

Monoclonal antibodies against these major glycoproteins participated to different extents in virus neutralization and antibody- and complement-mediated (AbC') cytolysis of virus-infected cells. In addition, purified gI and gIII stimulated the production of monospecific antibodies in rabbits, which neutralized viral infectivity and mediated immune lysis of virus-infected cells (van Drunen Littel-van den Hurk and Babiuk, 1985). These individual glycoproteins also stimulated antibody production in cattle infected with whole virus, suggesting that these glycoproteins may be important in mediating protective immune responses against BHV-1 (van Drunen Littel-van den Hurk and Babiuk, 1986b).

Our objectives in this project were to: 1) test the immunogenicity and protective capacity of affinity-purified BHV-1 glycoproteins to prevent respiratory disease in cattle, 2) test the immunogenicity and protective capacity of BHV-1 glycoproteins produced by recombinant vacciniavirus, 3) develop an ELISA to differentiate animals vaccinated with individual glycoproteins from those carrying virus.

III. Experimental Methods

Cells and Virus

Strains P8-2 and 108 of BHV-1 were propagated in Georgia bovine kidney cells as described previously (Babiuk et al., 1975). For virus challenge of animals strain 108 was used, whereas for glycoprotein isolation the Cooper strain was used.

Bacteria

A culture of Pasteurella haemolytica (biotype A, serotype 1) was prepared as described previously (Bielefeldt Ohmann and Babiuk, 1985). In each case, the bacterial challenge was in the log phase of growth and had a titer of 1 to 2 X 10^9 CFU/ml.

Preparation of Monoclonal Antibody Immunoadsorbent columns

Monoclonal antibodies against gI, gIII and gIV were produced as described previously (van Drunen Littel-van den Hurk et al., 1984). Clones 1D6, 1E11, and 3D9S which recognize gI, gIII and gIV, respectively, were selected to prepare immunoadsorbent columns.

Purification of IgG fractions of monoclonal antibodies was carried out using protein A-Sepharose CL-4B (Pharmacia Montreal, Quebec). The purified IgG was linked to activated Affigel-10 (Bio-Rad Laboratories, Mississauga, Ontario) at 5 mg protein/ml gel, according to the manufacturer's instructions.

Purification of glycoproteins

Glycoproteins gI, gIII and gIV were purified from virus-infected cell lysate as previously described (van Drunen Littel-van den Hurk and Babiuk, 1985). Twenty-four hours postinfection, at a m.o.i. of 1, cultures were harvested and centrifuged at 1000 rpm to obtain infected cell pellets. Cells were resuspended in 1% Nonidet-P40(NP-40) and 1% sodium deoxycholate (DOC) in 0.10 M Tris-hydrochloride, 0.15 M NaCl, pH 7.5) and used as starting material for purification.

Immunoadsorbent columns with specificities for gI, gIII or gIV, were prepared. After passage of the sample over the column in sample application buffer, the column was exchanged with 1 vol of fresh sample application buffer prior to washing with 2 vol of wash buffer [100 mM Tris, 500 mM NaCl, 1% NP-40 (pH 7.5)]. The wash buffer was displaced from the column with 2 vol of water prior to elution of the specifically bound antigen with 500 mM triethylamine. The eluted fractions were monitored by removing 5-50 ul/collected fraction and performing a nonquantative Bradford assay. Those fractions that contained protein were then directly concentrated for further analysis. The column was requilibrated in sample application buffer for reuse or stored in sample application buffer plus 0.02% thimersol. Columns prepared, used, and stored in this way have retained significant activity for almost a year.

Production of Recombinant Vacciniavirus Expressing BHV-1 gI or gIII

The complete coding sequence of BHV-1 was excised from a subclone of psD 106 (Mayfield et al., 1983) and inserted into the BAM H1 site of pGS 20 (Moss et al., 1983). This construction placed the BHV-1 gI gene sequence

immediately downstream of the vaccinia 7.5 kd promotor, which is flanked by vaccinia's thymidine kinase gene sequences. The construction was transfected into BSC-40 cells infected with vaccinia (strain Wyatt or strain WR) and recombinant virus was selected by passage into thymidine kinase negative 43 cells growing in bromodeoxyuridine media. The progeny of individual plaques was screened for expression of gI by ELISA using polyclonal or monoclonal antibodies specific for BHV-1 gI.

Recombinant vacciniavirus expressing BHV-1 gIII was produced in a similar manner except the complete coding sequence of gIII was derived from a subclone of pSD113 (Mayfield et al., 1983).

Immunization

Purified glycoproteins were mixed with avridine (N,N-dioctadecyl-N,N-bis) (2-hydroxylethylpropanediamine) as follows: 7 ml of glycoprotein (50 ug/ml), 2.8 ml of avridine (6 mg/ml), 30 ul of Tween 80, and 4.7 ml of Intralipid. Avridine was dissolved in ethanol prior to mixing with Tween 80 and Intralipid. The entire mixture was mixed by vortexing prior to mixing with the antigens. Groups of five animals each were immunized with a total of 100 ug of gI, gIII and gIV individually or in various combinations in a 2-ml volume. Twenty-one days later animals were boosted and then challenged 3 weeks after booster immunization. Control unvaccinated calves were immunized with avridine (adjuvant alone). A further control group was immunized with a commercial killed virus vaccine as recommended by the manufacturer. Animals were bled at 10-day intervals for assessment of antibody responses.

Experimental Challenge

Following immunization, animals were transported into an isolation pen and examined clinically, and rectal temperatures were recorded and blood samples were collected for various immunological assays to establish baseline immunological activity. The calves were then individually exposed to an aerosol of BHV-1, followed 4 days later with P. haemolytica. In each case, the aerosol was generated by a DeVilbiss Nebulizer, Model 65 (DeVilbiss, Barry, Ontario, Canada). Treatment was for 4 min in the case of the virus and 5 min with P. haemolytica as described previously (Bielefeldt Ohmann and Babiuk, 1985).

Enzyme-linked Immunosorbent Assay (ELISA)

In order to determine the antibody response of cattle immunized with purified glycoproteins, the ELISA was performed essentially as described previously (van Drunen Littel-van den Hurk et al., 1984). However, affinity-purified peroxidase-conjugated rabbit anti-bovine IgG (Zymed) at a dilution of 1:3000 was used as the detecting antibody.

Neutralization Test

The neutralization titers of the bovine sera were determined as described previously. To determine complement-enhanced neutralization, guinea pigs serum (1:40 final dilution) was added to the virus-antibody mixture. The titers were expressed as the reciprocal of the highest dilution of antibody which caused a 50% reduction of plaques relative to the virus control.

ADCC Assay

ADCC assays were performed in microtiter plates as described previously (Babiuk et al., 1975). The ratio of effector cells (polymorphonuclear cells) to target cells (BHV-1-infected, ^{51}Cr-labeled GBK cells) was 50:1. Controls consisted of BHV-1 serum or target with polymorphonuclear cells in the absence of antibody.

Clinical Evaluation

The clinical evaluations were performed at the same time each day by two independent investigators who were uninformed about the specific treatments of the individual animals. The parameters evaluated included depression, appetite, fever, conjunctivitis, rhinitis, mouth-breathing, tracheitis, and pneumonia. In each case a score of 0 was assigned to healthy animals. Clinical scores of 1-4 were assigned to sick animals for each individual parameter as follows: 4, severe; 3, marked; 2, moderate; 1, mild. Total clinical scores for each animal are the sums of scores for each parameter.

IV. Results and Discussion

1) Immunogenicity and Protective Capacity of Purified BHV-1 glycoproteins

Immune Responses to Purified Glycoproteins

Previously it was shown that small-scale purification of individual glycoproteins by immunosorbent columns resulted in pure preparations which retained antigenicity as determind by their ability to induce antibodies in rabbits and to react in Western blot assays (van Drunen Littel-van den Hurk and Babiuk, 1985). Similar results were obtained in this study using preparative columns (data not shown). Since the glycoproteins were pure and retained their antigenicity following purification by immunoadsorbent chromatography, they were tested for their ability to induce protective immune responses in cattle. Figure 1 indicates that within 10 days of immunization, all of the individual glycoproteins or combinations thereof induced detectable serum neutralizing titers. Following a booster immunization 21 days later, there was a further increase in the level of serum neutralizing antibodies induced by the glycoproteins. Highest responses were present in those animals immunized with gIV. In contrast, animals immunized with a commercial killed BHV-1 vaccine produced marginal antibody titers within 10 days of immunization. These antibody levels decreased to preimmunization levels by 20 days after immunization. Following a second immunization with the commercial vaccine, antibody titers were boosted to approximately the level observed 10 days postimmunization with the purified glycoprotein. In no case did the placebo-vaccinated animals develop any immune responses.

To measure the specificity of the immune response, the serum from each animal was tested by an ELISA using individual glycoproteins as the antigens. Animals immunized with gI only reacted in the ELISA when gI antigen was used to coat the plate. In contrast, those animals that were immunized with gIII only reacted with gIII coated plates. Similarly, animals immunized with gIV only recognized gIV (Figs. 2A,B,C). These results also indicate, similar to Fig. 1, that the animals immunized with gIV had higher titers than did animals immunized with the other glycoproteins.

Since the sera from animals immunized with glycoproteins were specific

for the individual glycoproteins used for immunization and could neutralize virus infectivity in vitro, attempts were made to determine whether any one of the individual glycoproteins could induce antibody capable of participating in ADCC. The results of Fig. 3 indicate that ADCC titers, although higher than SN titers, do parallel the serum neutralizing titers. Thus, animals immunized with gIV had higher ADCC titers than did animals immunized with the other glycoproteins, those immunized with gI being marginal in killing.

Protection Studies

Prior to challenge, all animals were healthy and had a normal rectal temperature. However, within 48 hr postinfection animals started exhibiting a rise in temperature (Fig. 4). Temperature responses continued to increase until they reached peak levels 4-7 days post-BHV-1 infection. In each case, animals within the groups immunized with the glycoproteins exhibited much lower temperature responses than did the placebo or animals immunized with the commercial whole virus vaccine. In addition to temperature responses, a variety of other parameters of respiratory distress were assessed. Based on our clinical scoring system (Babiuk et al., 1985) a clinical score of 10 or greater was set to indicate severe respiratory disease, which under field conditions would result in isolation of the animal and treatment to prevent pneumonia and subsequent death. Figure 5 indicates the total number of sick days (clinical score greater than 10) for the five animals within each individual treatment group. Animals immunized with individual glycoproteins or combinations thereof exhibited fewer days of morbidity than did the whole virus- or placebo-immunized animals.

Since the individual glycoproteins provided relatively good protection against severe respiratory disease, attempts were made to determine whether intramuscular immunization with individual glycoproteins had any effect on the extent of virus shedding from the nasal passages. Figure 6 indicates that all five of the placebo-treated animals shed virus for the entire observation period, beginning from Day 2 to Day 10 post-BHV-1 challenge. In contrast, animals immunized with the glycoproteins shed virus for a significantly fewer number of days ($P<0.005$ Fisher exact test). Once again, animals immunized with the commercial vaccine shed virus for more days than did animals immunized with individual glycoproteins (not significantly different from controls).

In the present model mortality rates of nonimmunized animals generally range from 40 to 80% (Babiuk et al., 1985). Figure 7 indicates that the mortality rate of placebo-immunized animals in this particular experiment was 60%, whereas it was 40% in those immunized with the commercial vaccine. However, none of the animals immunized with the individual or glycoprotein combinations died.

Discussion

After immunoadsorbent chromatography, pure preparations of each of the proteins were obtained and used for immunizing cattle. All three glycoproteins proved to be immunogenic in cattle and induced protective immune responses. Sera obtained from animals immunized with the individual glycoproteins were able to neutralize virus infectivity and participate in antibody-dependent cell cytotoxicity and complement-mediated lysis of infected cells.

At present, it appears that all three individual glycoproteins used in

this study is equivalent with respect to induction of protective immunity against virulent challenge. Furthermore, it does not appear that any of the glycoproteins act in a synergistic manner with respect to induction of protective immunity. However, to confirm that this is indeed the case, further experiments will have to be conducted wherein various dilutions of individual glycoproteins will be used as immunogens to determine whether low levels of immunity produced by small quantities of the individual glycoproteins will act in a synergistic manner. We have recently demonstrated that monoclonal antibodies directed against different epitopes can act in a synergistic manner and thereby increase the neutralizing titer of the sera over that observed when the individual monclonal antibodies were used (van Drunen Littel-van den Hurk et al., 1985). If this were the case, then it may be possible to use very small quantities of individual purified glycoproteins with appropriate adjuvants to induce protection against virulent BHV-1 infection.

It is interesting to note that the level of immunity as determined by antibody levels in the serum and protection from virulent challenge was much greater in animals immunized with the individual glycoproteins than are those immunized with the commercially available killed whole virus vaccine. The reason for this increased protection is not readily apparent. However, it could be related to the concentration of antigen that is presented in the killed vaccine. The lowest dose of antigen we used in any of the vaccines was 33 ug of each individual glycoprotein. Whether a similar quantity of antigen is presented in the killed vaccine remains to be determined. Alternatively, it is possible that some of the antigens that are present in the whole viral vaccine are in actual fact immunosuppressive and therefore reduce the animals' ability to respond to the protective glycoproteins. If this is the case, then it is even more important to produce subunit vaccines against herpesviruses which do not contain immunosuppressive proteins. It is interesting to note that following BHV-1 infections in vivo the animal's immune response is also suppressed. Whether this is related to the viruses' ability to replicate in specific cells of the immune system or whether there is an indirect effect mediated by individual proteins or glycoproteins remains to be determined. Investigations in this regard are presently in progress.

2) Immunogenicity and Protective Capacity of BHV-1 Glycoproteins Produced by Recombinant Viruses

The genes for glycoproteins gI and gIII have each been inserted into the vacciniavirus genome. Several in vitro experiments indicated that the products of the BHV-1 glycoprotein genes, inserted into vaccinia, were similar to or identical with the glycoproteins synthesized by BHV-1. However, the levels of glycoproteins gI and gIII, produced by vacciniaviruses were approximately 6-fold lower than those produced by BHV-1. Moreover, neither of the two recombinant vacciniaviruses were able to stimulate the production of glycoprotein-specific antibodies in rabbits or to confer protection to lethal BHV-1/P. haem. challenge in cattle. Higher quantities of recombinant vacciniavirus or, alternatively, increased expression of the inserted glycoprotein genes may be needed before the vacciniavirus can be used as an effective vaccine.

3) Development of ELISA

Microtiter plates were coated with individual glycoproteins gI, gIII and gIV. Sera from vaccinated animals were tested using the ELISA. The specificity of the test was demonstrated by the observation that animals immunized with a particular glycoprotein reacted only with that glycoprotein, but not with any of the others. However, animals vaccinated with conventional vaccine reacted with all glycoproteins (Fig. 2). Consequently, by using this test it is possible to differentiate between animals vaccinated with individual glycoprotein(s) and those immunized with a conventional vaccine or naturally infected with a field strain.

Impact

Respiratory disease in cattle causes major economic losses annually to the Saskatchewan and Western Canadian cattlemen. One of the major initiators of the disease is BHV-1, commonly called IBRV. In addition to causing respiratory disease, this virus also causes abortion in cattle and can be transmitted in semen. Therefore, countries free of BHV-1 refuse to accept semen from animals that have antibodies to the virus, because these animals could be potential carriers of BHV-1.

In the present study we demonstrated that each of the BHV-1 glycoproteins gI, gIII and gIV individually or in various combinations, induced the production of antibodies in cattle, which neutralized viral infectivity and participated in lysis of virus-infected cells. Most importantly, these glycoproteins induced protection against challenge with virulent BHV-1. These data suggest that the individual glycoproteins may each be useful candidates for a subunit vaccine. Using a sensitive ELISA, we were able to diffentiate between animals vaccinated with individual glycoproteins and those immunized with a conventional vaccine or individually infected with a field strain.

The benefits of having a subunit vaccine and the ELISA will be of value to the beef producer, dairy industry and purebred breeder interested in export of pedigree stock or semen.

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Figure 1 Serum neutralizing antibody responses of animals immunized with BHV-1 glycoproteins. Animals were immunized with the various glycoproteins gI (♦), gIII (+), gIV (■), gI + gIII (◇), gI + gIV (□), gIII + gIV (▲), gI + gIII + g IV (X) a commercial killed vaccine (△) or placebo (-) and boosted 3 weeks later. Titers were determined by a 50% endpoint using 100 PFU of BHV-1 virus.

Figure 2 ELISA titers in serum of calves immunized with BHV-1 glycoproteins A) plate coated with gI antigen. Responses of animals immunized with gI, gI + gIII, gI + gIV, gIII, gIV, a commercial vaccine, placebo B) plates coated with gIII antigen. Responses of animals immunized with gI, gIII, gIV, gI + gIII, gIII + gIV, a commercial vaccine and placebo. C) plates coated with gIV antigen. Response of animals immunized with gI, gIII, gIV, gI + gIV, gIII + gIV, a commerical vaccine or a placebo.

Figure 3 ADCC titers of sera obtained from calves immunized with BHV-1 glycoproteins gIII, gIV, gI + gIII, gI + gIV, gIII + gIV, gI + gIII + gIV, a commercial vaccine.

Figure 4 Temperature responses of immunized animals following challenge with virulent BHV-1. Mean of all animals immunized with different glycoproteins (35 animals) (■), animals immunized with a commercial vaccine (□) or placebo (X).

Figure 5 Effect of immunization with BHV-1 glycoproteins on clinical response of calves to BHV-1/P. haemolytica challenge. Number above the bars indicate the total number of animal days in each group, exhibiting a clinical score of 10 or more on any specific observation over a 10-day observation period.

Figure 6 Virus shedding from the nasal passage of animals. Nasal samples were collected for 8 days (day 2-10 post-challenge). Numbers above the bars indicate the total number of animal days each group shed virus. Number of animals/group was 5. Maximum possible days of shedding was 40.

Figure 7 Mortality of animals following challenged with BHV-1/P. haemolytica. Values above the bars indicate number of animals dying. Total of the animals in each group was 5.

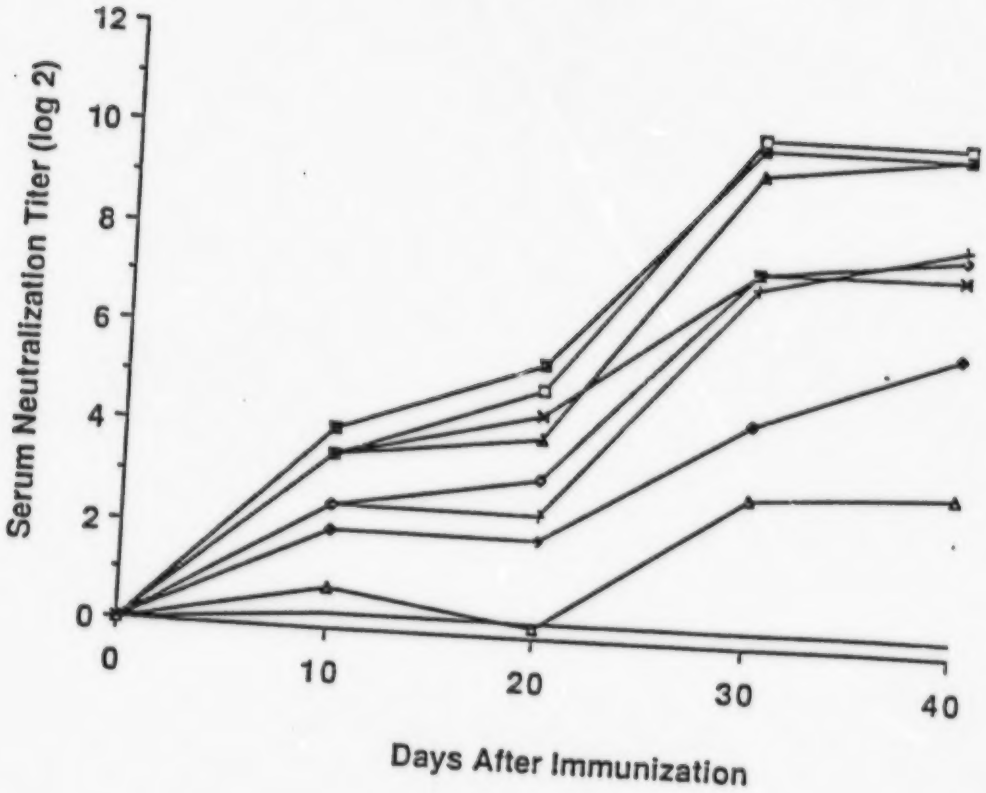
Serum Neutralization Titer (log 2)
12
10
8
6
4
2
0
0
10
20
30
40
Days After Immunization

Figure 1

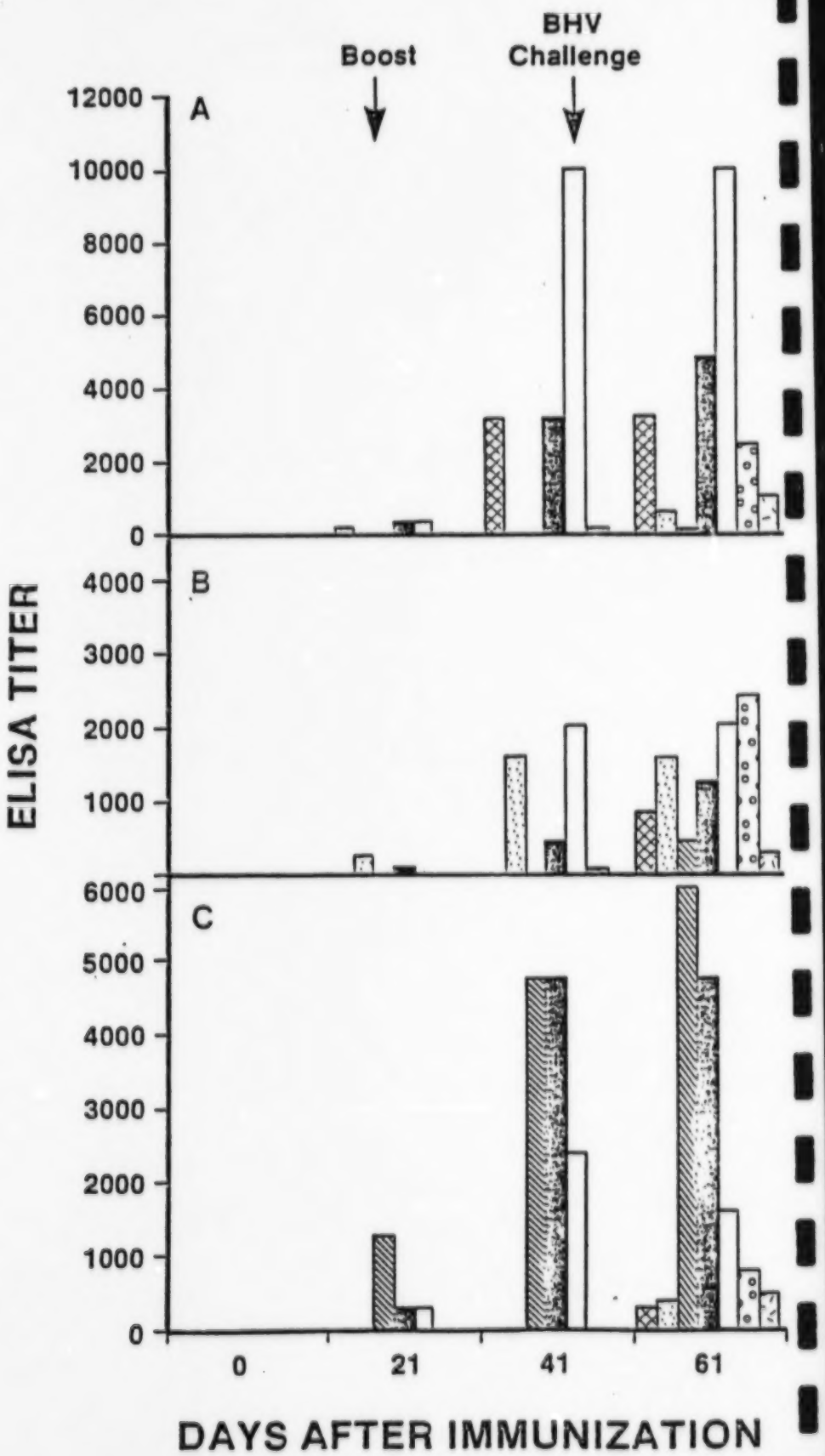
Boost
BHV Challenge
A
B
C
12000
10000
8000
6000
4000
2000
0
4000
3000
2000
1000
6000
5000
4000
3000
2000
1000
0
0
21
41
61
ELISA TITER
DAYS AFTER IMMUNIZATION

Figure 2

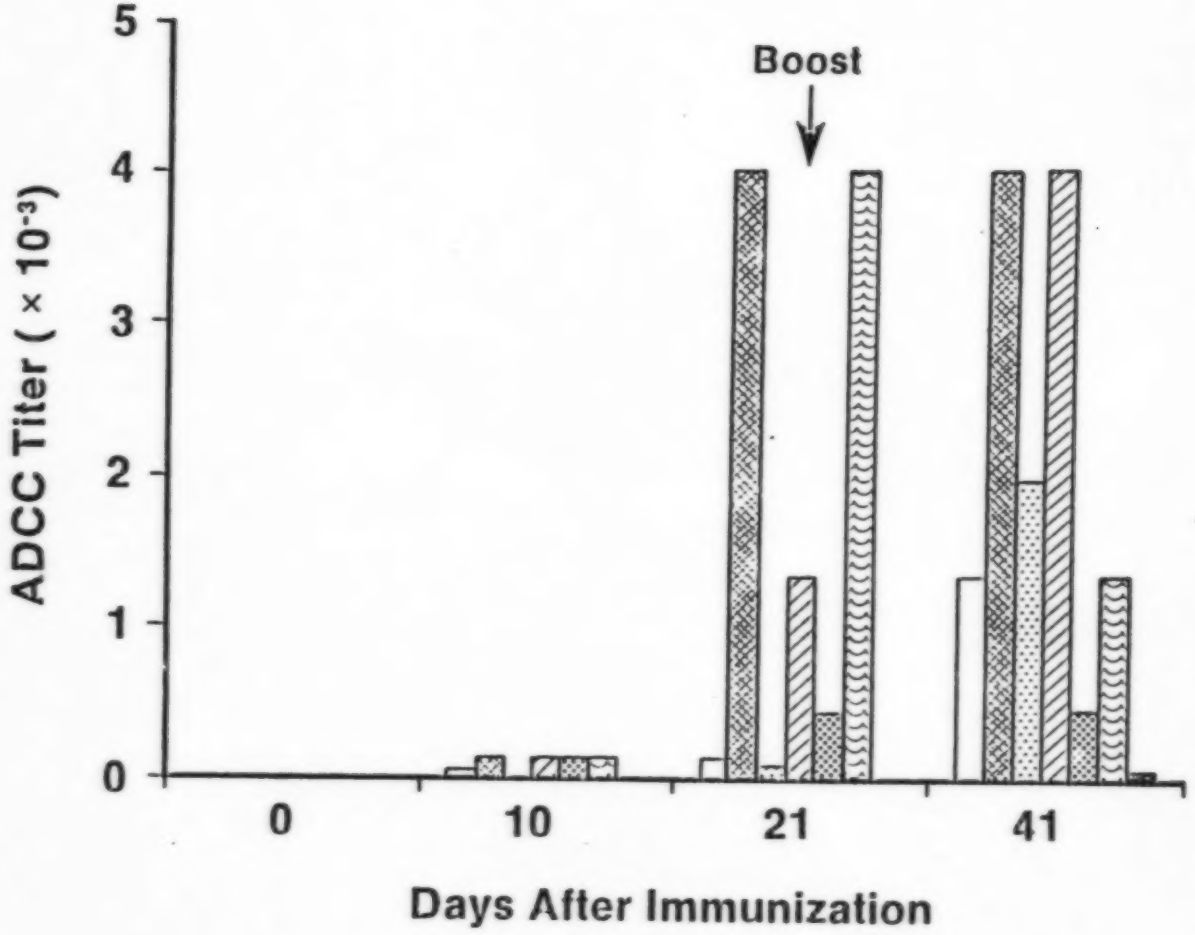
Boost
5
4
3
2
1
0
ADCC Titer (× 10⁻³)
0
10
21
41
Days After Immunization

Figure 3

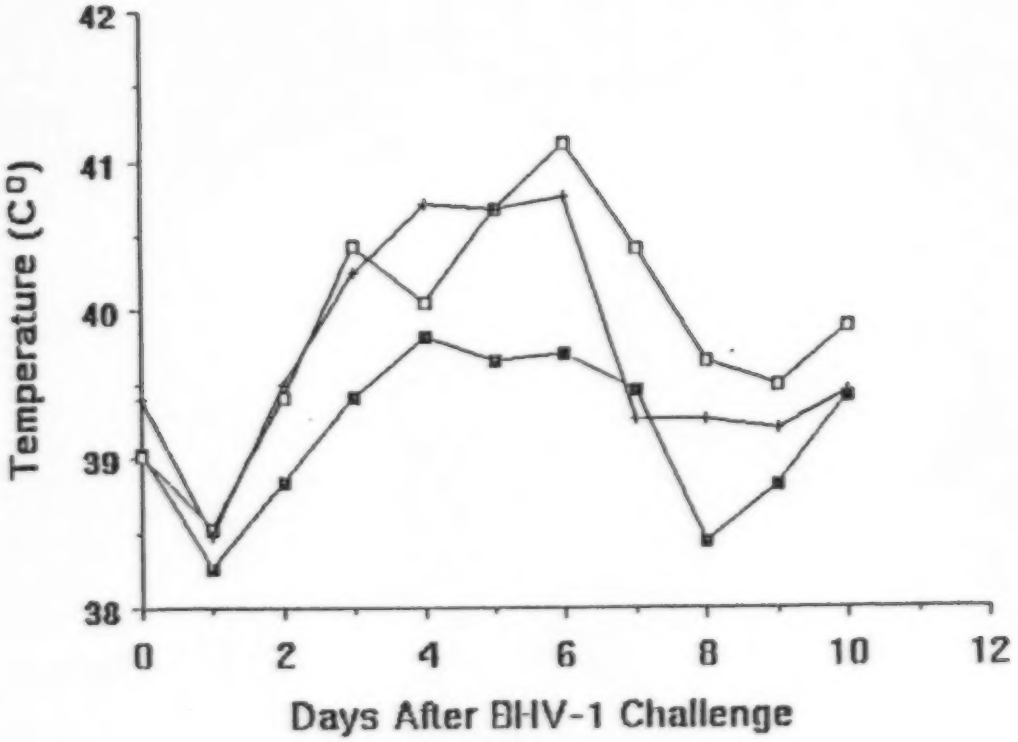
42
41
40
39
38
Temperature (C°)
0
2
4
6
8
10
12
Days After BHV-1 Challenge

Figure 4

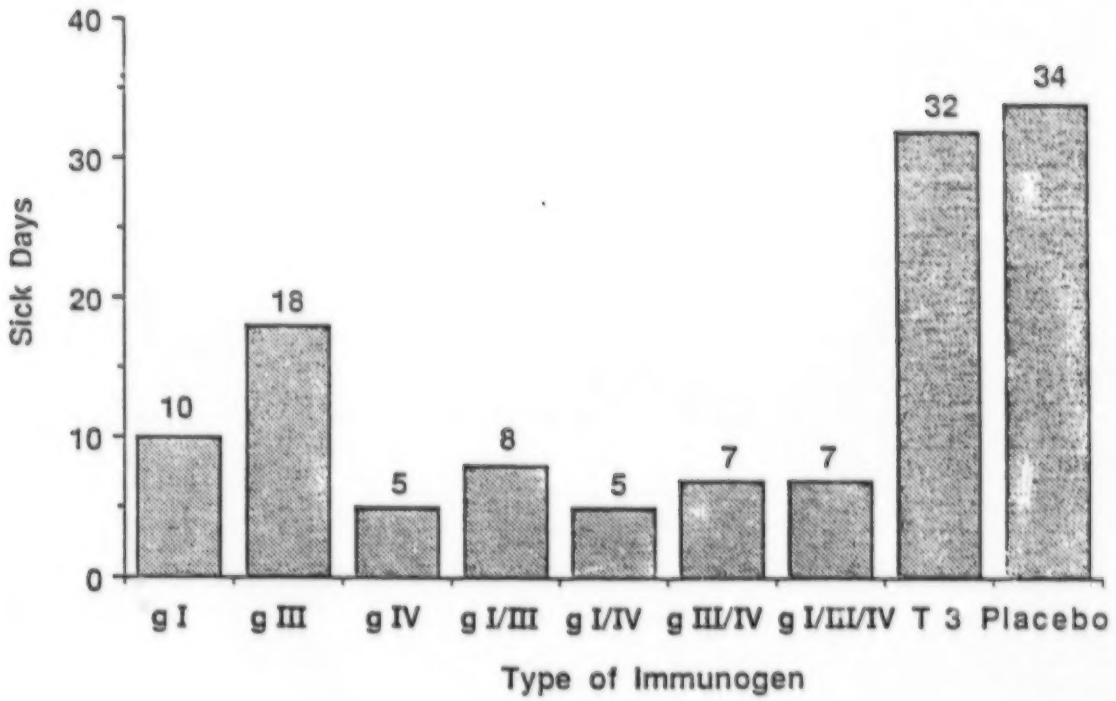
Sick Days
40
30
20
10
0
10
18
5
8
5
7
7
32
34
g I
g III
g IV
g I/III
g I/IV
g III/IV
g I/III/IV
T 3
Placebo
Type of Immunogen

Figure 5

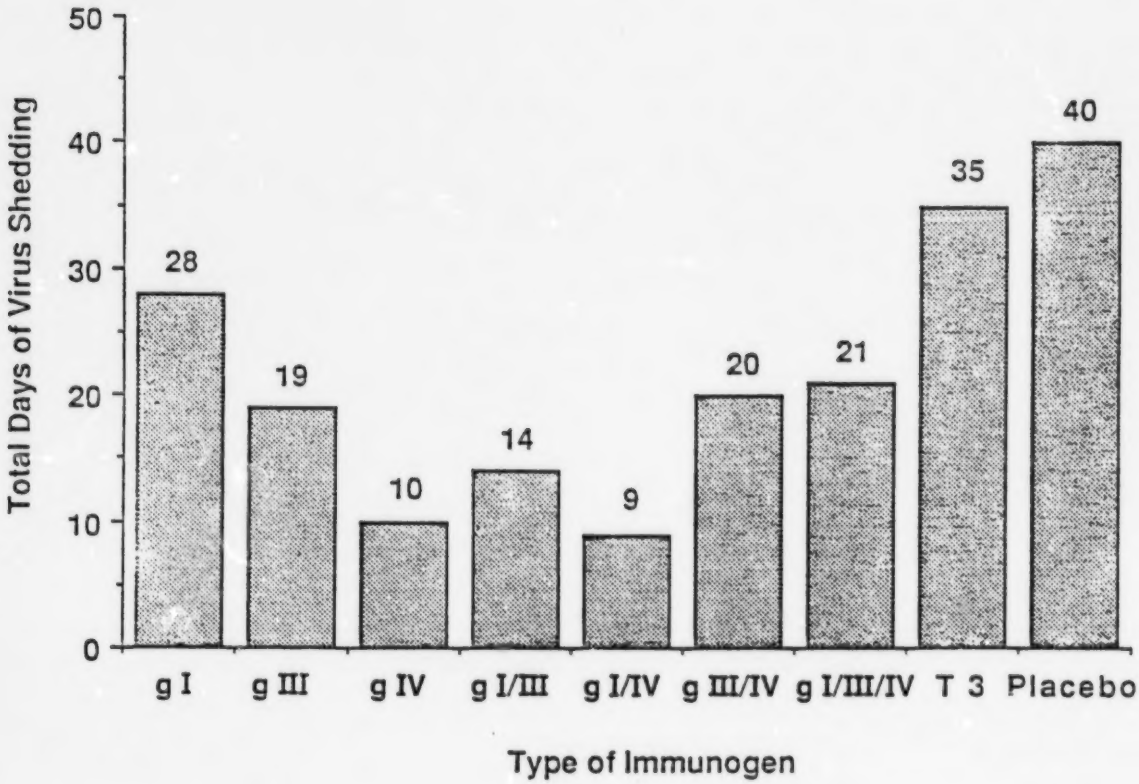
Total Days of Virus Shedding
50
40
30
20
10
0
28
19
10
14
9
20
21
35
40
g I
g III
g IV
g I/III
g I/IV
g III/IV
g I/III/IV
T 3
Placebo
Type of Immunogen

Figure 6

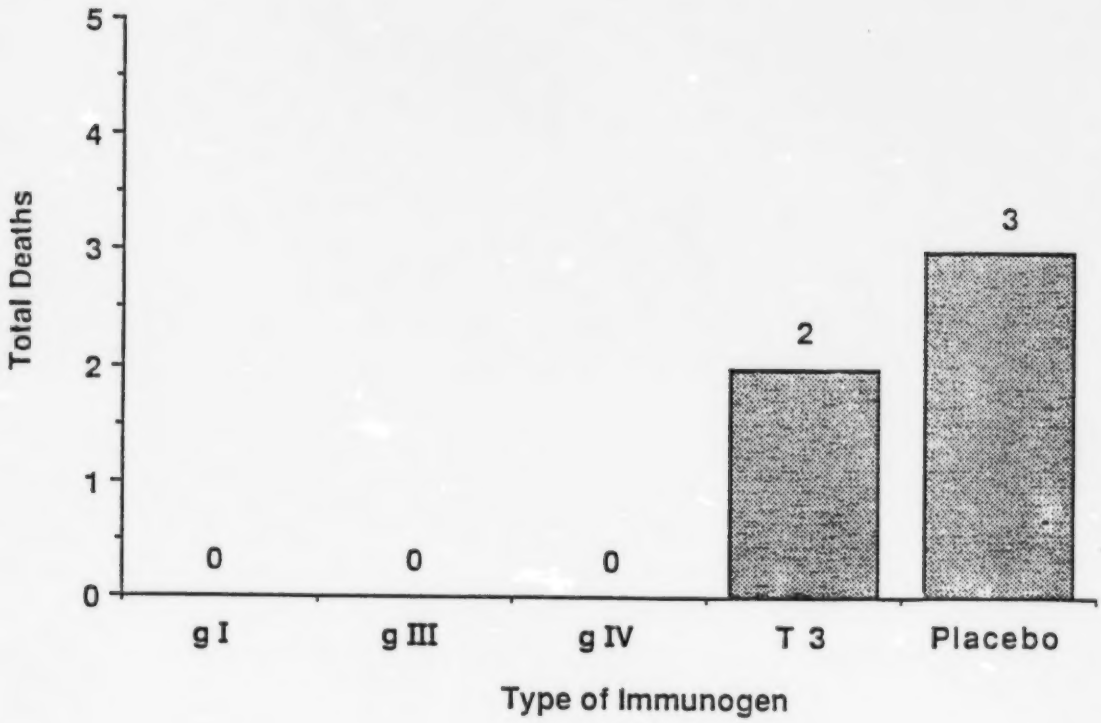
Total Deaths
5
4
3
2
1
0
0
0
0
2
3
g I
g III
g IV
T 3
Placebo
Type of Immunogen

Figure 7

Research Publications in Scientific Journals

Babiuk, L.A., L'Italien, J., van Drunen Littel-van den Hurk, S., Zamb, T., Lawman, M.P.J., Hughes, G., and Gifford, G. (1987). Protection of cattle from bovine herpesvirus type-1 (BHV-1) infection by immunization with individual viral glycoproteins. Virology 158. In press.

van Drunen Littel-van den Hurk, S., Zamb, T., Hughes, G., and Babiuk, L.A. (1987). Characterization of vaccinia recombinants expressing bovine herpesvirus-1 (BHV-1) glycoproteins gI or gIII. Manuscript in preparation.

Contributed Papers in Published Meetings and Abstracts and Posters at Meetings

van Drunen Littel-van den Hurk, S., L'Italien, J., Lawman, M.P.J., Gifford, G., Zamb, T., Hughes, G., and Babiuk, L.A. (1986). Protection of cattle from lethal bovine herpesvirus-1 (BHV-1)/Pasteurella haemolytica (P. Haem.) infection by immunization with affinity-purified BHV-1 glycoproteins. Abstracts of Eleventh International Herpesvirus Workshop. Leeds. UK.

van Drunen Littel-van den Hurk, S., Zamb, T., Hughes, G., and Babiuk, L.A, (1987). Characterization of vaccinia recombinants expressing bovine herpesvirus-1 (BHV-1) glycoproteins gI or gIII. VIIth International Congress of Virology, Edmonton, Canada.